UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF
CHEMICAL SAFETY
AND POLLUTION
PREVENTION

MEMORANDUM

DATE: January 25, 2013

SUBJECT: Efficacy Review for Alice,
EPA Reg. No. 74559-T;
DP Barcode: D406670;

FROM: Lorilyn M. Montford LM 2/4/13
Efficacy Evaluation Team
Antimicrobials Division (7510P)

THRU: Emily Mitchell, Chief EM 2/4/13
Product Science Branch
Antimicrobials Division (7510P)

TO: Marshall Swindell, PM 33/Demson Fuller
Regulatory Management Branch II
Antimicrobials Division (7510P)

APPLICANT: Virox Technologies, Inc.
2770 Coventry Road
Oakville, Ontario L6H 6R1
Canada

FORMULATION FROM LABEL:

Active Ingredient(s)	% by wt.
Hydrogen Peroxice	0.5%
Other Ingredients	99.5%
Total	100.0%

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I BACKGROUND

The product, Alice (EPA File Symbol 74559-T), is a new product. The applicant requested to register the product for use as a soft surface spot sanitizer and deodorizer for carpets and other soft fabrics in household, commercial, and institutional environments. [The proposed label makes a claim for one-step cleaning.] Studies were conducted at ATS Labs, located at 1285 Corporate Center Drive, Suite 110, in Eagan, MN 55121.

This data package contained a letter from the applicant's representative to EPA (dated September 12, 2012), EPA Form 8570-4 (Confidential Statement of Formula), EPA Form 8570-27 (Formulator's Exemption Statement), EPA Form 8570-34 (Certification with Respect to Citation of Data), EPA Form 8570-35 (Data Matrix), three studies (MRID 48945901 through 48945903) and one supplemental efficacy discussion document, Statements of No Data Confidentiality Claims for all three studies, and the proposed label.

II USE DIRECTIONS

The product is designed for spot sanitizing and deodorizing soft surfaces such as: carpets made of polypropylene/olefin, nylon and polyester; upholstery and other soft fabric surfaces such as cotton fabrics, canvas, shower curtains, fabric curtains and draperies, blankets, bedding (for pets), car seats, couches, and mattresses.

Directions on the proposed label provide the following information regarding use of the product as a spot sanitizer for carpets: Spray a light even coating on soiled area until damp. Gently blot area with a clean, damp, color-safe cloth. Repeat as needed (for stubborn stains), Let air dry. (Vacuum.) (Deep clean following your machines guide.)

Directions on the proposed label provide the following information regarding use of the product as a spot sanitizer: Spray a light even coating on area until damp. Let air dry, Vacuum.

Directions on the proposed label provide the following information regarding use of the product on soft surfaces other than carpet: Spray a light even coating on soiled area [fabric] until damp. Allow area to remain wet for 1 minute. Gently blot area with a clean, damp, color-safe cloth. Repeat as needed (for stubborn stains or heavy fabrics). Let air dry.

III AGENCY STANDARDS

EFFICACY DATA REQUIREMENTS FOR CARPET SANITIZERS:

Three product samples representing 3 separate batches, one of which is at least 60 days old, must be tested against *Staphylococcus aureus* ATCC 6538 and *Enterobacter aerogenes* ATCC 13048 with 2 different types of representative synthetic carpeting, such as acrylic and polypropylene tufted-loop types. If the application is intended for hospitals or medical institutions, the product must also be tested against *Pseudomonas aeruginosa* ATCC 15442. If the product is also intended for use on wool carpeting, an additional representative sample of wool carpet must be tested; otherwise, the label must bear a disclaimer for use on wool. All carpet samples tested must be fully identified by the pile fiber type, pile yarn weight of finished carpet, pile density, and tuft height. Adequate controls must demonstrate that bacteriostatic agents in the carpet pile or backing do not yield false-negative data which interfere with the test results.

Supplemental Claims

An antimicrobial agent identified as a "one-step" disinfectant or as effective in the presence of organic soil must be tested for efficacy with an appropriate organic soil load, such as 5 percent serum.

Note: There are currently no accepted Agency Standards for soft surface spot sanitization. Applicants interested in claims for soft surface sanitization are requested to submit protocols supporting this use.

IV COMMENTS ON THE SUBMITTED EFFICACY STUDIES

1. MRID 48945901 "Carpet Sanitizer," Test Organisms: *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048), for Alice, by Jill Rhume. Study conducted at ATS Labs. Study completion date – April 12, 2012. Project Number A12889.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. BHC-052-18A, BHC-052-18B and BHC-052-18C), one of which is at least 60 days old of the product, Alice, were tested using the ATS Protocol (Protocol # BLL01021512.CSAN) set up according to the Agency Methodology for Carpet Sanitizers. The test substance was received ready to use (RTU). The test organisms were transferred daily on Nutrient A slants for ≥ 3 but ≤ 30 transfers. The growth was washed from a 24+2 hour Nutrient Agar slant using 5.0 mL of phosphate buffer dilution water (PBDW). This growth suspension was aspirated and added to 99.0 mL PBDW. A 2.0 mL aliquot of each suspension was then added to sufficient Nutrient Agar B bottles. The inoculum was evenly distributed within the bottles and the excess inoculum was removed. The bottles were incubated agar side down for 18-24 hours at 25-30°C for *Enterobacter aerogenes* and 35-37°C for *Staphylococcus aureus*. Following incubation, a 3.0 mL aliquot of PBDW and approximately 15-20 sterile glass beads were added to each bottle to suspend the growth. The growth suspension was removed and filtered through sterile gauze pre-wetted with 1.00 mL of PBDW. A 0.40 mL aliquot of fetal bovine serum (FBS) was added to 7.6 mL of each broth culture to yield a 5% fetal bovine serum organic load. The sponsor supplied the carriers (8 x 12 inch pieces of nylon and polypropylene/olefin carpet) used in testing. The carpet was fastened to a mounting tray and was autoclave sterilized for ≥ 20 minutes prior to use in testing. Each cut square was inoculated with 100 µL of the prepared test culture using a calibrated pipettor. The inoculum was spread over the upper surface of the carpet using a sterile loop. The inoculated carpet was dried in an incubator at 35-37°C for 60 minutes with sterile foil loosely covering the carpet. After drying the test substance was applied by spraying each carrier at a distance of 4-6 inches using four sprays (approximately 3 g). Each carpet carrier was then scrubbed for approximately 30 seconds using approximately 30 circular clockwise strokes and approximately 30 counterclockwise strokes. A circular area of pile approximately 3 inches in diameter around the center of each carrier was scrubbed using this treatment. Moderate to heavy pressure was applied downward on the brush to work the solution to the base of the pile. A new sterile brush was used for each carpet square. The treated and scrubbed carpet remained at room temperature (21°C), covered, for 60 minutes. Following the 60 minute exposure time, each carpet piece was removed from the larger carpet piece. The carpet carriers were then transferred to individual vessels containing 100 mL of neutralizer broth and 10 stainless steel penicylinders representing the 10^0 dilution. The vessels were shaken vigorously for one minute using an orbital shaker at approximately 200 RPM in order to free the bacteria from the carpet

fibers. Ten fold serial dilutions were prepared for both *Staphylococcus aureus* and *Enterobacter aerongenes.* All test plates and controls were incubated for 48±4 hours at 25-30°C for *Enterobacter aerogenes* and at 35-37°C for *Staphylococcus aureus.* Following incubation, the subcultures were visually examined for growth. Controls included those for unscrubbed population, scrubbed population, neutralization confirmation, purity, organic sterility, carrier sterility and neutralizer sterility.

2. MRID 48945902 "Carpet Sanitizer," Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (6538), for Alice, by Jill Rhume. Study conducted at ATS Labs. Study completion date – June 26, 2012. Project Number A13407.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. BHC-052-18A, BHC-052-18B and BHC-052-18C), one of which is at least 60 days old of the product, Alice, were tested using the ATS Protocol (Protocol # BLL01042012.CSAN) set up according to the Agency Methodology for Carpet Sanitizers. The test substance was received ready to use (RTU). The test organisms were transferred daily on Nutrient A slants for ≥ 3 but ≤ 30 transfers. The growth was washed from a 24+2 hour Nutrient Agar slant using 5.0 mL of phosphate buffer dilution water (PBDW). A 2.0 mL aliquot of each suspension was then added to sufficient Nutrient Agar B bottles. The inoculum was evenly distributed within the bottles and the excess inoculum was removed. The bottles were incubated agar side down for 18-24 hours at 25-30°C for *Enterobacter aerogenes* and 35-37°C for *Staphylococcus aureus.* Following incubation, a 3.0 mL aliquot of PBDW and approximately 15-20 sterile glass beads were added to each bottle to suspend the growth. The growth suspension was removed and filtered through sterile gauze pre-wetted with 1.00 mL of PBDW. Cultures were incubated for 48-54 hours at 35-37°C. A 0.20 mL aliquot of fetal bovine serum (FBS) was added to 3.8 mL of each broth culture to yield a 5% fetal bovine serum organic load. ATS Labs supplied the carriers (8 x 12 inch pieces of polyester carpet) used in testing. The carpet was fastened to a mounting tray and was autoclave sterilized for ≥ 20 minutes prior to use in testing. Each cut square was inoculated with 100 µL of the prepared test culture using a calibrated pipettor. The inoculum was spread over the upper surface of the carpet using a sterile loop. The inoculated carpet was dried in an incubator at 35-37°C for 60 minutes with sterile foil loosely covering the carpet. After drying the test substance was applied by spraying each carrier at a distance of 4-5 inches using four sprays (approximately 3 g/carrier). Each carpet carrier was then scrubbed for approximately 30 seconds using approximately 30 circular clockwise strokes and approximately 30 counterclockwise strokes. A circular area of pile approximately 3 inches in diameter around the center of each carrier was scrubbed using this treatment. Moderate to heavy pressure was applied downward on the brush to work the solution to the base of the pile. A new sterile brush was used for each carpet square. The treated and scrubbed carpet remained at room temperature (20°C), covered, for 60 minutes. Following the 60 minute exposure time, each carpet piece was removed from the larger carpet piece. The carpet carriers were then transferred to individual vessels containing 100 mL of neutralizer broth and 10 stainless steel penicylinders representing the 10^{0} dilution. The vessels were shaken vigorously for one (1) minute using an orbital shaker at approximately 200 RPM in order to free the bacteria from the carpet fibers. Ten fold serial dilutions were prepared for both *Staphylococcus aureus* and *Enterobacter aerogenes.* All test plates and controls were incubated for 48±4 hours at 25-30°C for *Enterobacter aerogenes* and at 35-37°C for *Staphylococcus aureus.* Following incubation, the subcultures were visually examined for growth. Controls included those for unscrubbed population, scrubbed population, neutralization

confirmation, purity, organic sterility, carrier sterility and neutralizer sterility.

3. MRID 48945903 "Standard Test Method for Efficacy of Sanitizers Recommended for Soft Non-Food Contact Surfaces (Modification for Spray Product Application)"" Test Organisms: *Enterobacter aerogenes* (ATCC 13048) and *Staphylococcus aureus* (6538), for Alice, by Jill Rhume. Study conducted at ATS Labs. Study completion date – April 12, 2012. Project Number A12890.

This study was conducted against *Staphylococcus aureus* (ATCC 6538) and *Enterobacter aerogenes* (ATCC 13048). Three lots (Lot Nos. BHC-052-18A, BHC-052-18B and BHC-052-18C), one of which is at least 60 days old of the product, Alice, were tested using the Standard Test Method Recommended for Soft Non-Food Contact Surfaces (Modified for Spray Products). The test substance was received ready to use (RTU). From a stock slant, an initial tube (10 mL) of culture broth was inoculated. This culture was termed "the initial broth suspension". From this initial broth suspension, a minimum of three daily transfers using 1 loopful of culture into 10mL of culture media was performed on consecutive days prior to use in testing procedure. A 48-54 hour culture was vortex-mixed and allowed to settle for $\geq$15 minutes. The upper 2/3rds of the culture was removed and transferred to a sterile vessel for use in testing. The test culture was thoroughly mixed. A 0.20 aliquot of fetal bovine serum was added to 3.80 mL of each prepared culture to yield a 5% fetal bovine serum organic soil load.
To prepare the 100% polyester fabric, a scouring solution was prepared by adding approximately 0.4032 grams Na_2CO_3 and 0.4199 grams of Triton X-100 to approximately 0.8mL of deionized water. To prepare the fabric containing 80 x80 thread/inch plain cotton weave, a scouring solution was prepared by adding approximately 6.0331 grams Na_2CO_3 and 6.1295 grams of Triton X-100 to approximately 12L of deionized water. Approximately 1206.88 grams of test fabric was added to each 12L volume of scouring solution. Each solution was allowed to boil for approximately 60 minutes. The fabric was removed and rinsed through a rinsing procedure. The fabric was allowed to air dry. The carriers were cut from the fabric to a size of approximately 1 in. x 1 in. and were autoclave sterilized. Afterwards, each carrier was placed into a sterile Petri dish prior to use in testing. Each of the five test carriers were sprayed with the test substance using staggered intervals Carriers were sprayed at a distance of 4 inches + 1 inch using 2 sprays and were allowed to expose at room temperature (21°C) and 56% relative humidity for 1 minute, 3 minutes, and 5 minutes. Following exposure each carrier was transferred to 20 mL of neutralizer using identical staggered intervals. Following neutralization of the test carriers, the excess liquid in each Petri dish was transferred to the neutralizer jar containing the matching carrier. The carriers were vortex-mixed. Within 30 minutes of neutralization, for *Staphylococcus aureus*, duplicate 1.00 mL aliquots of the neutralized solution and duplicate 1.00 mL aliquots of a ten-fold serial dilution were plated onto the recovery agar plate medium. The same procedure was done for *Enterobacter aerogenes*. The *Staphylococcus aureus* plates were incubated at 35-37°C for 48$\pm$4 hours. The *Enterobacter aerogenes* plates were incubated for 48$\pm$4 hours at 25-30°C. Following incubation, subcultures were visually examined. Controls included those for carrier population, sterility, neutralizer sterility, purity, organic soil load and neutralization confirmation.

V RESULTS

(MRID 48945901) for Polypropylene/Olefin Carpet

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})
BHC-052-18A (≥ 60 days old)	*E. aerogenes* (ATCC 13048)	7.33	2.14×10^7	78.7% (0.66 log_{10})
BHC-052-18B	*E. aerogenes* (ATCC 13048)	5.77	5.89×10^5	99.4% (2.22 $_{log10}$)
BHC-052-18C	*E. aerogenes* (ATCC 13048)	6.01	1.02×10^6	98.9% (1.98 log_{10})

(MRID 48945901) for Nylon Carpet

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})
BHC-052-18A (≥ 60 days old)	*E. aerogenes* (ATCC 13048)	<4.26	$<1.82 \times 10^4$	99.9% (>3.61 log_{10})
BHC-052-18B	*E. aerogenes* (ATCC 13048)	<4.01	$<1.02 \times 10^4$	99.9% (>3.86 $_{log10}$)
BHC-052-18C	*E. aerogenes* (ATCC 13048)	<2.78	$<6.03 \times 10^2$	99.9% (>5.09 log_{10})

(MRID 48945901) for Polypropylene/Olefin Carpet

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})
BHC-052-18A (≥ 60 days old)	*S. aureus* (ATCC 6538)	<4.24	1.74 x 104	99.9% (>3.10 log_{10})
BHC-052-18B	*S. aureus* (ATCC 6538)	5.00	1.00×10^5	99.5% (2.34 $_{log10}$)
BHC-052-18C	*S. aureus* (ATCC 6538)	<4.31	2.04 x 104	>99.9% (>3.03 log_{10})

(MRID 48945901) for Nylon Carpet

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})
BHC-052-18A (≥ 60 days old)	*S. aureus* (ATCC 6538)	<3.70	<5.01 x 103	99.9% (>4.03 log_{10})
BHC-052-18B	*S. aureus* (ATCC 6538	<2.00	1.00×10^2	99.9% (>5.73 log_{10})
BHC-052-18C	*S. aureus* (ATCC 6538)	<2.48	3.02×10^2	99.9% (5.25 log_{10})

(MRID 48945902) for Polyester Carpet

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})
BHC-052-18A (≥ 60 days old)	*E. aerogenes* (ATCC 13048)	<4.56	$<3.63 \times 10^4$	99.9%
	S. aureus (ATCC 6538)	4.57	3.72×10^4	>99.9%
BHC-052-18B	*E. aerogenes* (ATCC 13048)	<4.04	1.10×10^4	99.9%
	S. aureus (ATCC 6538)	3.33	2.14×10^3	>99.9%
BHC-052-18C	*E. aerogenes* (ATCC 13048)	<4.49	$<3.09 \times 10^4$	99.9%
	S. aureus (ATCC 6538)	3.25	1.78×10^3	>99.9%

(MRID 48945903) for Plain Cotton Weave and Polyester Fabric(Soft Surface Sanitizer Test)

Lot Number	Organism	Average CFU/Carrier (Log_{10})	Geometric Mean	Percent Reduction (Log_{10})

BHC-052-18A (≥ 60 days old) 1, 3 and 5 minutes	***E. aerogenes* and *S. aureus***	**1.30**	**<2.00 x 10^1**	**>99.9%**
BHC-052-18B 1, 3 and 5 minutes	***E. aerogenes* and *S. aureus***	**1.30**	**<2.00 x 10^1**	**>99.9%**
BHC-052-18C 1, 3 and 5 minutes	***E. aerogenes* And *S. aureus***	**1.30**	**<2.00 x 10^1**	**>99.9%**

VI CONCLUSIONS

1. The submitted efficacy data (MRID 48945901) support the use of the product, Alice, as a soft surface sanitizer against the following microorganisms on nylon carpet in the presence of a 5% organic soil load for a 60 minute contact time:

Staphylococcus aureus	MRID 48945901
Enterobacter aerogenes	MRID 48945901

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms. Purity controls were reported as pure. Sterility controls did not show growth.

The submitted efficacy data (MRID 48945901) **do not support** the use of the product, Alice, as a soft surface sanitizer against the following microorganism on polypropylene/olefin carpet in the presence of a 5% organic soil load for a 60 minute contact time:

Staphylococcus aureus	MRID 48945901
Enterobacter aerogenes	MRID 48945901

Complete killing was not observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive growth of the microorganisms.

2. The submitted efficacy data (MRID 48945902) support the use of the product, Alice, as a soft surface sanitizer against the following microorganisms on polyester carpet in the presence of a 5% organic soil load for a 60 minute contact time:

Staphylococcus aureus	MRID 48945902
Enterobacter aerogenes	MRID 48945902

Complete killing was observed in the subcultures of the required number of carriers tested against the required number of product lots. At least one of the product lots tested was at least 60 days old at the time of testing. Neutralization confirmation testing showed positive

growth of the microorganisms. Purity controls were reported as pure. Sterility controls did not show growth.

3. The submitted efficacy data (MRID 48945903) support the use of the product, Alice, as a soft surface sanitizer against the following microorganisms on polyester fabric and plain cotton weave fabric in the presence of a 5% organic soil load for a contact time of 1 minute, 3 minutes and 5 minutes:

Staphylococcus aureus	MRID 48945903
Enterobacter aerogenes	MRID 48945903

Important Note:

4. The registrant submitted a supplemental efficacy discussion document (MRID 48945904) on soft surface and carpet sanitizer studies. The information submitted was provided as a supplement to the three studies submitted. The purpose of the written supplement was to provide the Agency with an explanation of the relationship between the sponsor (Bissell Homecare, Inc, and the registrant, Virox Technologies, Inc. The information was not an additional study to be reviewed. The supplemental information was submitted to summarize their findings.

VII RECOMMENDATIONS

1. The proposed label claims that the product, Alice, is an effective soft surface sanitizer for use on nylon, and polyester carpet against the following microorganisms in the presence of a 5% organic soil load for a contact time of 60 minutes:

Staphylococcus aureus
Enterobacter aerogenes

These claims are acceptable as they are supported by the submitted data.

2. The proposed label claims that the product, Alice, is an effective soft surface sanitizer for use on polyester fabric and plain cotton weave fabric against the following microorganisms in the presence of a 5% organic soil load for a contact time of 1 minute, 3 minutes and 5 minutes:

Staphylococcus aureus
Enterobacter aerogenes

These claims are acceptable as they are supported by the submitted data.

3. The following revisions must be made to the proposed label:

- Please add a statement to the proposed label that states that the product is not recommended for use on carpets made from polypropylene/olefin fibers.
- On page 1 of the proposed label, delete "bacteria/killer/destroyer/eliminator".
- On page 5 of the proposed label, remove statements that say, "Controls allergens or Controls most allergens". These terms imply pesticide prevention of the product.

- On page 5 of the proposed label, please include "ATCC numbers" for the organisms listed. ATCC numbers can also be listed either on the master label (as optional text) with the listing organisms claimed or on the final page of the master label (as optional text)

- On page 6 of the proposed label, remove the statement, "Controls the growth of bacteria". This statement is misleading and also implies prevention.

- On bottom of page 7 of the proposed label, remove the statement, "Goes beyond normal cleaning". This statement implies heightened efficacy and is not allowed.

- On page bottom of page 11 of the proposed label, remove all statements that state, "Gentle on your carpet"; "Gentle clean", "Gentle on"; "Safe and Gentle for your carpet, and "Safe for your carpet", "Gentle on your soft surfaces", and "Safe for use on.." These terms suggest safety claims of a pesticide.

- On page 11 of the proposed label, remove the statement, "No (harmful) residue" and "No (harsh) chemical residue".

- On page 11 of the proposed label, remove the statement, "No resoiling".

- On page 11 of the proposed label, remove the statement, "Safe for all carpets". This statement is misleading since the data did support use on all types of carpet fibers.

- On page 11 of the proposed label, remove the statement, "Carefully designed..." Statements that suggest a product possess unique characteristics is not allowed.

- On page 11 of the proposed label, remove the statement, "Leaves carpet safe for children and pets when used as directed." Safety claims of a pesticide are not allowed.

- On page 12 of the proposed label, remove the word, "(harmful)" from the statement, "Leaves no (harmful) residue". Again, safety claims are not allowed.

- On page 12 of the proposed label, remove the statement, "Simple Power".

- On page 12 of the proposed label, remove the statement, "Gentle mist dries in minutes". The term "gentle" suggests safety claims by the pesticide product.